

WHAT IS CLAIMED IS:

1. A method for synthesizing isopentenyl pyrophosphate in a host microorganism,
wherein the method comprises introducing into the host microorganism a plurality of
5 heterologous nucleic acid sequences, each coding for a different enzyme in the mevalonate
pathway for producing isopentenyl pyrophosphate.

2. The method of claim 1, wherein the plurality of heterologous nucleic acid sequences
is integrated into the chromosome of the host microorganism.

3. The method of claim 1, wherein the plurality of heterologous nucleic acid sequences
is contained in at least one extrachromosomal expression vector.

4. The method of claim 3, wherein the plurality of heterologous nucleic acid sequences
is present in a single expression vector.

5. The method of claim 4, wherein the single expression vector contains the nucleotide
sequence SEQ ID NO 7.

6. The method of claim 3, wherein each heterologous nucleic acid sequence is contained
within a different expression vector.

7. The method of claim 3, wherein at least two of the heterologous nucleic acid
sequences are contained in a single expression vector.

8. The method of claim 3, wherein some of the heterologous nucleic acid sequences are
contained in a first expression vector, and the remainder of the sequences, in a second
expression vector.

9. The method of claim 8, wherein the first expression vector contains the nucleotide sequence SEQ ID NO 8, and the second expression vector includes the nucleotide sequence contained in SEQ ID NO 9.

5 10. The method of claim 1, wherein the plurality of heterologous nucleic acid sequences comprises:

- 10 a) a DNA fragment coding for an enzyme capable of condensing two molecules of acetyl-CoA to acetoacetyl-CoA;
- b) a DNA fragment coding for an enzyme capable of condensing acetoacetyl-CoA with acetyl-CoA to form HMG-CoA;
- c) a DNA fragment coding for an enzyme capable of converting HMG-CoA to mevalonate;
- d) a DNA fragment coding for an enzyme capable of phosphorylating mevalonate to mevalonate 5-phosphate;
- 15 e) a DNA fragment coding for an enzyme capable of converting mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and
- f) a DNA fragment coding for an enzyme capable of converting mevalonate 5-pyrophosphate to isopentenyl pyrophosphate.

20 11. The method of claim 10, wherein the plurality of individual heterologous nucleic acid sequences comprises:

- a) the nucleotide sequence of SEQ ID NO 1;
- b) the nucleotide sequence of SEQ ID NO 2;
- c) the nucleotide sequence of SEQ ID NO 3;
- 25 d) the nucleotide sequence of SEQ ID NO 4;
- e) the nucleotide sequence of SEQ ID NO 5; and
- f) the nucleotide sequence of SEQ ID NO 6.

30 12. The method of claim 1, wherein the isopentenyl pyrophosphate is recovered from the host microorganism.

13. The method of claim 1, wherein the isopentenyl pyrophosphate is further modified to provide an isoprenoid.

14. The method of claim 13, wherein the plurality of heterologous nucleic acid sequences further comprises:

g) a DNA fragment coding for an enzyme capable of converting isopentenyl pyrophosphate to dimethylallyl pyrophosphate.

15. The method of claim 13, wherein the isoprenoid is selected from the group consisting of a monoterpene, sesquiterpene, diterpene, sesterterpene, triterpene, tetraterpene, and a steroid.

16. The method of claim 15, wherein the isoprenoid is a monoterpene.

17. The method of claim 16, wherein the monoterpene is selected from the group consisting of limonene, citranellool, and geraniol.

18. The method of claim 15, wherein the isoprenoid is a sesquiterpene.

19. The method of claim 18, wherein the sesquiterpene is selected from the group consisting of periplanone B, artemisinin, ginkgolide B, forskolin, and farnesol.

20. The method of claim 15, wherein the isoprenoid is is a diterpene.

21. The method of claim 20, wherein the diterpene is selected from the group consisting of casbene and paclitaxel.

22. The method of claim 1, wherein the host microorganism is a prokaryote.

23. The method of claim 22, wherein the prokaryote is *Escherichia coli*.

24. A method for synthesizing isopentenyl pyrophosphate in a host microorganism,
wherein the method comprises introducing into the host microorganism an intermediate in the
mevalonate pathway and at least one heterologous nucleic acid sequence, each said sequence
coding for an enzyme in the mevalonate pathway necessary for converting the intermediate into
isopentenyl pyrophosphate.

25. The method of claim 24, wherein a plurality of heterologous nucleic acid sequences
is introduced into the host microorganism.

26. The method of claim 25, wherein the plurality of heterologous nucleic acid
sequences is integrated into the chromosome of the host microorganism.

27. The method of claim 25, wherein the plurality of heterologous nucleic acid
sequences is contained in at least one extrachromosomal expression vector.

28. The method of claim 27, wherein the plurality of heterologous nucleic acid
sequences is present in a single expression vector.

29. The method of claim 28, wherein the expression vector includes the nucleotide
sequence contained in SEQ ID NO 9.

30. The method of claim 25, wherein the intermediate is DL-mevalonate and the
plurality of heterologous sequences comprises:

a) a DNA fragment coding for an enzyme capable of phosphorylating mevalonate to
mevalonate 5-phosphate;

b) a DNA fragment coding for an enzyme capable of converting mevalonate 5-phosphate
to mevalonate 5-pyrophosphate; and

c) a DNA fragment coding for an enzyme capable of converting mevalonate 5-pyrophosphate to isopentenyl pyrophosphate.

31. The method of claim 30, wherein the plurality of individual heterologous nucleic acid sequences comprises:

- a) the nucleotide sequence of SEQ ID NO 4;
- b) the nucleotide sequence of SEQ ID NO 5; and
- c) the nucleotide sequence of SEQ ID NO 6.

32. The method of claim 24, wherein the isopentenyl pyrophosphate is recovered from the host microorganism.

33. The method of claim 25, wherein the plurality of heterologous nucleic acid sequences further comprises:

- g) a DNA fragment coding for an enzyme capable of converting isopentenyl pyrophosphate to dimethylallyl pyrophosphate.

34. The method of claim 24, wherein the isopentenyl pyrophosphate is further modified to provide an isoprenoid.

35. The method of claim 34, wherein the isoprenoid is selected from the group consisting of a monoterpene, sesquiterpene, diterpene, sesterterpene, triterpene, tetraterpene, and a steroid.

36. The method of claim 35, wherein the isoprenoid is a monoterpene.

37. The method of claim 36, wherein the monoterpene is selected from the group consisting of limonene, citranellol, and geraniol.

38. The method of claim 35, wherein the isoprenoid is a sesquiterpene.

39. The method of claim 38, wherein the sesquiterpene is selected from the group consisting of periplanone B, artemisinin, ginkgolide B, forskolin, and farnesol.

40. The method of claim 35, wherein the isoprenoid is a diterpene.

41. The method of claim 40, wherein the diterpene is selected from the group consisting of casbene and paclitaxel.

42. The method of claim 24, wherein the host microorganism is a prokaryote.

43. The method of claim 42, wherein the prokaryote is *Escherichia coli*.

44. An isolated DNA fragment coding for the enzymes in the mevalonate pathway for producing isopentenyl pyrophosphate.

45. The isolated DNA fragment of claim 44, comprising the nucleotide sequence of SEQ ID NO 7.

46. An expression vector comprising the DNA fragment of claim 44.

47. The expression vector of claim 46, wherein the DNA fragment comprises the nucleotide sequence of SEQ ID NO 7.

48. A host cell transformed with the expression vector of claim 46.

49. An isolated DNA fragment coding for a fraction of the enzymes in the mevalonate pathway for producing isopentenyl pyrophosphate comprising the nucleic acid sequences comprised of:

- a) a DNA fragment coding for an enzyme capable of condensing two molecules of acetyl-CoA to acetoacetyl-CoA;
- b) a DNA fragment coding for an enzyme capable of condensing acetoacetyl-CoA with acetyl-CoA to form HMG-CoA; and
- 5 c) a DNA fragment coding for an enzyme capable of converting HMG-CoA to mevalonate.

10 50. The DNA fragment of claim 49, comprising the nucleotide sequence of SEQ ID NO 8.

15 51. An expression vector comprising the DNA fragment of claim 49.

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15 52. The expression vector of claim 51, comprising the nucleotide sequence of SEQ ID NO 8.

15 53. A host cell transformed with the expression vector of claim 51.

20 54. An isolated DNA fragment coding for a fraction of the enzymes in the mevalonate pathway for producing isopentenyl pyrophosphate comprising the nucleic acid sequences comprised of:

- a) a DNA fragment coding for an enzyme capable of phosphorylating mevalonate to mevalonate 5-phosphate;
- b) a DNA fragment coding for an enzyme capable of converting mevalonate 5-phosphate to mevalonate 5-pyrophosphate; and
- 25 c) a DNA fragment coding for an enzyme capable of converting mevalonate 5-pyrophosphate to isopentenyl pyrophosphate.

30 55. The DNA fragment of claim 54, comprising the nucleotide sequence of SEQ ID NO 9.

56. An expression vector comprising the DNA fragment of claim 54.

57. The expression vector of claim 55, comprising the nucleotide sequence of
SEQ ID NO 9.

58. A host cell transformed with the expression vector of claim 56.

59. A host cell transformed with a first expression vector containing some of the
heterologous nucleic acid sequences coding for enzymes in the mevalonate pathway for
producing isopentenyl pyrophosphate contained in a first expression vector, and the remainder
of the sequences, in a second expression vector.

60. The host cell of claim 59, wherein the first expression vector contains the nucleotide
sequence of SEQ ID NO 8 and the second expression vector contains the nucleotide sequence of
SEQ ID NO 9.

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